FORM PTO-1082

Date: January 20, 1998
Case Docket No. 20220-0169
Express Mail No. EM 181 247 764 US

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Transmitted herewith for filing is the patent application of
Inventors: Edith Mathiowitz, Jules S. Jacob, Donald E. Chickering, III, and Kathleen J. Pekarek
For: Preparation of Multiwall Polymeric Microcapsules from Hydrophilic Polymers

Enclosed are:

☒ Copies of the Consent to Correct Named Inventors and Certificates under 37 CFR § 3.73(b).

☒ Assignments of the invention to Massachusetts Institute of Technoloty and Children Medical Center corporation

☐ A certified copy of a _____ application.

☒ Copy of executed Declaration.

☐ A verified statement to establish small entity status under 37 CFR 1.9 and 37 CFR 1.27

☒ Request for Filing a Divisional Application under 37 CFR §1.53(b).

The filing fee has been calculated as shown below:

	(Col. 1)	(Col. 2)	SMALL ENTITY			OTHER THAN A SMALL ENTITY	
FOR:	NO. FILED	NO. EXTRA	RATE	FEE	OR	RATE	FEE
BASIC FEE				$395	OR		$790
TOTAL CLAIMS	18-20 =	0	x 11	$	OR	x 22 =	$
INDEP CLAIMS	2-3 =	0	x 41 =	$	OR	x 82 =	$
☐ MULTIPLE DEPENDENT CLAIM PRESENTED			x135 =	$	OR	x270 =	$
			TOTAL	$	OR	TOTAL	$790

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- ☐ The issue fee set in 37 CFR 1.18 at or before mailing of the Notice of Allowance, pursuant to 37 CFR 1.311(b).
- ☒ Any filing fees under 37 CFR 1.16 for the presentation of extra claims.

Respectfully submitted,

Patrea L. Pabst, Reg. No. 31,284

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Keith T. Paige, Linda G. Cima, Charles A. Vacanti, and Anthony Atala

Serial No: Divisional of 08/056,140

Express Mail No.:
EM 1810 247 764 US

Filed: January 20, 1998

Filing Date:
January 20, 1998

For: *TISSUE FORMATION BY INJECTING A CELL-POLYMERIC SOLUTION THAT GELS IN VIVO* (As Amended)

BOX PATENT APPLICATION
Assistant Commissioner of Patents
Washington, D.C. 20231

REQUEST FOR FILING A DIVISIONAL APPLICATION UNDER 37 C.F.R. § 1.53(b)

Sir:

This is a request for the filing a divisional Application under 37 C.F.R. § 1.53(b), of pending prior application Serial No. 08/056,140, filed April 30, 1993, by Keith T. Paige, Linda G. Cima, Charles A. Vacanti and Anthony Atala, for "*TISSUE FORMATION BY INJECTING A CELL-POLYMERIC SOLUTION THAT GELS IN VIVO*" (As Amended).

I hereby verify that the attached papers are a true copy of what is shown in my records to be the above-identified prior application, including the declaration as originally filed and the subsequently filed executed Declaration. The application includes 1 page of Abstract, 17 pages of Specification, 3 pages of claims, an unexecuted Declaration for Patent Application, and the subsequently executed Declaration for Patent Application.

Also enclosed are copies of an Assignment to Children's Medical Center Corporation from Keith T. Paige, Joseph P. Vacanti, recorded at reel 6626, frame 0675, on July 6, 1993; an Assignment to Children's Medical Center Corporation from Anthony Atala and Charles A. Vacanti, recorded at reel 6909, frame 0292, on March 21, 1994; and an Assignment to Massachusetts Institute of Technology from Linda G. Cima, recorded at reel 6909, frame 0296, on March 21, 1994. A copy of the Consent to Correct Named Inventors and Certificates under 37 C.F.R. § 3.73(b) from Massachusetts Institute of Technology and Children's Medical Center Corporation; and return postcard are also enclosed.

The inventorship for the claims in the divisional application is the same as in the prior application.

The attorney of record in the prior application is Patrea L. Pabst, Registration No. 31,284, and the Power of Attorney appears in the original papers in the prior application.

A check in the amount of $ 790.00 is enclosed to cover the filing fee for the divisional application. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 01-2507. A duplicate copy of this Request is enclosed.

This application is being filed on January 20, 1998, by mailing the application to Box Patent Application, Commissioner for Patents and Trademarks, Washington, D.C. 20231 via the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 C.F.R. § 1.10. The Express Mail Label No. EM 181 247 764 US appears in the heading of this paper, which is attached to the application, pursuant to 37 C.F.R. §1.10(b).

I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: January 20, 1998
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CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.10

I hereby certify that this REQUEST FOR FILING A DIVISIONAL APPLICATION UNDER 37 C.F.R. §1.53(b) and any documents referred to as attached therein are being deposited with the United States Postal Service on this date, January 20, 1998, in an envelope as "Express Mail Post Office to Addressee" service under 37 C.F.R § 1.10, Mailing Label Number EM 181 247 764 US addressed to Box Patent Application, Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Carol E. Terry

Date: January 20, 1998

APPLICATION

FOR

UNITED STATES LETTERS PATENT

BY

KEITH T. PAIGE

AND

JOSEPH P. VACANTI

INJECTABLE POLYSACCHARIDE-CELL COMPOSITIONS

INJECTABLE POLYSACCHARIDE-CELL COMPOSITIONS

Background of the Invention

The present invention is generally in the area of creating new tissues using polysaccharide hydrogel-cell compositions.

Craniofacial contour deformities, whether traumatic, congenital, or aesthetic, currently require invasive surgical techniques for correction. Furthermore, deformities requiring augmentation often necessitate the use of alloplastic prostheses which suffer from problems of infection and extrusion. A minimally invasive method of delivering additional autogenous cartilage or bone to the craniofacial skeleton would minimize surgical trauma and eliminate the need for alloplastic prostheses. If one could transplant via injection and cause to engraft large numbers of isolated cells, one could augment the craniofacial osteo-cartilaginous skeleton with autogenous tissue, but without extensive surgery.

Unfortunately, attempts to inject dissociated cells subcutaneously or to implant dissociated tissues within areas of the body such as the peritoneum have not been successful. Cells are relatively quickly removed, presumably by phagocytosis and cell death.

Cells can be implanted onto a polymeric matrix and implanted to form a cartilaginous structure, as described in U.S. Patent No. 5,041,138 to Vacanti, et al., but this requires surgical implantation of the matrix and shaping of the matrix prior to implantation to form a desired anatomical structure.

Accordingly, it is an object of the present invention to provide a method and compositions for injection of cells to form cellular tissues and cartilaginous structures.

It is a further object of the invention to provide compositions to form cellular tissues and cartilaginous structures including non-cellular material which will degrade and be removed to leave tissue or cartilage that is histologically and chemically the same as naturally produced tissue or cartilage.

Summary of the Invention

Slowly polymerizing, biocompatible, biodegradable hydrogels have been demonstrated to be useful as a means of delivering large numbers of isolated cells into a patient to create an organ equivalent or tissue such as cartilage. The gels promote engraftment and provide three dimensional templates for new cell growth. The resulting tissue is similar in composition and histology to naturally occurring tissue. In one embodiment, cells are suspended in a hydrogel solution and injected directly into a site in a patient, where the hydrogel hardens into a matrix having cells dispersed therein. In a second embodiment, cells are suspended in a hydrogel solution which is poured or injected into a mold having a desired anatomical shape, then hardened to form a matrix having cells dispersed therein which can be be implanted into a patient. Ultimately, the hydrogel degrades, leaving only the resulting tissue.

This method can be used for a variety of reconstructive procedures, including custom molding of cell implants to reconstruct three dimensional tissue defects, as well as implantation of tissues generally.

Detailed Description of the Invention

Techniques of tissue engineering employing biocompatible polymer scaffolds hold promise as a means of creating alternatives to prosthetic materials currently used in craniomaxillofacial surgery, as well as formation of organ equivalents to replaced diseased, defective, or injured tissues. However, polymers used to create these scaffolds, such as polylactic acid, polyorthoesters, and polyanhydrides, are difficult to mold and hydrophobic, resulting in poor cell attachment. Moreover, all manipulations of the polymers must be performed prior to implantation of the polymeric material.

Calcium alginate and certain other polymers can form ionic hydrogels which are malleable and can be used to encapsulate cells. In the preferred embodiment described herein, the hydrogel is produced by cross-linking the anionic salt of alginic acid, a carbohydrate polymer isolated from seaweed, with calcium cations, whose strength increases with either increasing concentrations of calcium ions or alginate. The alginate solution is mixed with the cells to be implanted to form an alginate suspension. Then, in one embodiment, the suspension is injected directly into a patient prior to hardening of the suspension. The suspension then hardens over a short period of time. In a second embodiment, the suspension is injected or poured into a mold, where it hardens to form a desired anatomical shape having cells dispersed therein.

Polymeric Materials.

The polymeric material which is mixed with cells for implantation into the body should form a hydrogel. A hydrogel is defined as a substance

formed when an organic polymer (natural or synthetic) is cross-linked via covalent, ionic, or hydrogen bonds to create a three-dimensional open-lattice structure which entraps water molecules to form a gel. Examples of materials which can be used to form a hydrogel include polysaccharides such as alginate, polyphosphazines, and polyacrylates, which are crosslinked ionically, or block copolymers such as Pluronics™ or Tetronics™, polyethylene oxide-polypropylene glycol block copolymers which are crosslinked by temperature or pH, respectively.

In general, these polymers are at least partially soluble in aqueous solutions, such as water, buffered salt solutions, or aqueous alcohol solutions, that have charged side groups, or a monovalent ionic salt thereof. Examples of polymers with acidic side groups that can be reacted with cations are poly(phosphazenes), poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers, such as sulfonated polystyrene. Copolymers having acidic side groups formed by reaction of acrylic or methacrylic acid and vinyl ether monomers or polymers can also be used. Examples of acidic groups are carboxylic acid groups, sulfonic acid groups, halogenated (preferably fluorinated) alcohol groups, phenolic OH groups, and acidic OH groups.

Examples of polymers with basic side groups that can be reacted with anions are poly(vinyl amines), poly(vinyl pyridine), poly(vinyl imidazole), and some imino substituted polyphosphazenes. The ammonium or quaternary salt of the polymers can also be formed from the backbone nitrogens or

pendant imino groups. Examples of basic side groups are amino and imino groups.

Alginate can be ionically cross-linked with divalent cations, in water, at room temperature, to form a hydrogel matrix. Due to these mild conditions, alginate has been the most commonly used polymer for hybridoma cell encapsulation, as described, for example, in U.S. Patent No. 4,352,883 to Lim. In the Lim process, an aqueous solution containing the biological materials to be encapsulated is suspended in a solution of a water soluble polymer, the suspension is formed into droplets which are configured into discrete microcapsules by contact with multivalent cations, then the surface of the microcapsules is crosslinked with polyamino acids to form a semipermeable membrane around the encapsulated materials.

Polyphosphazenes are polymers with backbones consisting of nitrogen and phosphorous separated by alternating single and double bonds. Each phosphorous atom is covalently bonded to two side chains ("R"). The repeat unit in polyphosphazenes has the general structure (1):

where n is an integer.

The polyphosphazenes suitable for cross-linking have a majority of side chain groups which are acidic and capable of forming salt bridges with di- or trivalent cations. Examples of preferred acidic side groups are carboxylic acid groups and sulfonic acid groups. Hydrolytically stable polyphosphazenes are formed of monomers having carboxylic acid side groups that are crosslinked by divalent or trivalent cations such as Ca^{2+} or Al^{3+}.

Polymers can be synthesized that degrade by hydrolysis by incorporating monomers having imidazole, amino acid ester, or glycerol side groups. For example, a polyanionic poly[bis(carboxylatophenoxy)]phosphazene (PCPP) can be synthesized, which is cross-linked with dissolved multivalent cations in aqueous media at room temperature or below to form hydrogel matrices.

Bioerodible polyphosphazines have at least two differing types of side chains, acidic side groups capable of forming salt bridges with multivalent cations, and side groups that hydrolyze under *in vivo* conditions, e.g., imidazole groups, amino acid esters, glycerol and glucosyl. The term bioerodible or biodegrable, as used herein, means a polymer that dissolves or degrades within a period that is acceptable in the desired application (usually *in vivo* therapy), less than about five years and most preferably less than about one year, once exposed to a physiological solution of pH 6-8 having a temperature of between about 25°C and 38°C. Hydrolysis of the side chain results in erosion of the polymer. Examples of hydrolyzing side chains are unsubstituted and substituted imidizoles and amino acid esters in which the group is bonded to the phosphorous atom through an amino linkage (polyphosphazene polymers in which both R groups are attached in this manner are known as polyaminophosphazenes). For polyimidazolephosphazenes, some of the "R" groups on the polyphosphazene backbone are imidazole rings, attached to phosphorous in the backbone through a ring nitrogen atom. Other "R" groups can be organic residues that do not participate in hydrolysis, such as methyl phenoxy groups or other

groups shown in the scientific paper of Allcock, et al., Macromolecule 10:824-830 (1977).

Methods for synthesis and the analysis of various types of polyphosphazenes are described by Allcock, H.R.; et al., Inorg. Chem. 11, 2584 (1972); Allcock, et al., Macromolecules 16, 715 (1983); Allcock, et al., Macromolecules 19, 1508 (1986); Allcock, et al., Biomaterials, 19, 500 (1988); Allcock, et al., Macromolecules 21, 1980 (1988); Allcock, et al., Inorg. Chem. 21(2), 515-521 (1982); Allcock, et al., Macromolecules 22, 75 (1989); U.S. Patent Nos. 4,440,921, 4,495,174 and 4,880,622 to Allcock, et al.; U.S. Patent No. 4,946,938 to Magill, et al.; and Grolleman, et al., J. Controlled Release 3, 143 (1986), the teachings of which are specifically incorporated herein by reference.

Methods for the synthesis of the other polymers described above are known to those skilled in the art. See, for example Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, E. Goethals, editor (Pergamen Press, Elmsford, NY 1980). Many polymers, such as poly(acrylic acid), are commercially available.

The water soluble polymer with charged side groups is crosslinked by reacting the polymer with an aqueous solution containing multivalent ions of the opposite charge, either multivalent cations if the polymer has acidic side groups or multivalent anions if the polymer has basic side groups. The preferred cations for cross-linking of the polymers with acidic side groups to form a hydrogel are divalent and trivalent cations such as copper, calcium, aluminum, magnesium, strontium, barium, and tin, although di-, tri- or tetra-functional organic cations such as alkylammonium salts, e.g., R_3N^+ -\/\/\/-$^+NR_3$ can also be used. Aqueous

solutions of the salts of these cations are added to the polymers to form soft, highly swollen hydrogels and membranes. The higher the concentration of cation, or the higher the valence, the greater the degree of cross-linking of the polymer. Concentrations from as low as 0.005 M have been demonstrated to cross-link the polymer. Higher concentrations are limited by the solubility of the salt.

The preferred anions for cross-linking of the polymers to form a hydrogel are divalent and trivalent anions such as low molecular weight dicarboxylic acids, for example, terepthalic acid, sulfate ions and carbonate ions. Aqueous solutions of the salts of these anions are added to the polymers to form soft, highly swollen hydrogels and membranes, as described with respect to cations.

A variety of polycations can be used to complex and thereby stabilize the polymer hydrogel into a semi-permeable surface membrane. Examples of materials that can be used include polymers having basic reactive groups such as amine or imine groups, having a preferred molecular weight between 3,000 and 100,000, such as polyethylenimine and polylysine. These are commercially available. One polycation is poly(L-lysine); examples of synthetic polyamines are: polyethyleneimine, poly(vinylamine), and poly(allyl amine). There are also natural polycations such as the polysaccharide, chitosan.

Polyanions that can be used to form a semi-permeable membrane by reaction with basic surface groups on the polymer hydrogel include polymers and copolymers of acrylic acid, methacrylic acid, and other derivatives of acrylic acid, polymers with pendant SO_3H groups such as sulfonated polystyrene, and polystyrene with carboxylic acid groups.

Sources of Cells.

Cells can be obtained directed from a donor, from cell culture of cells from a donor, or from established cell culture lines. In the preferred embodiments, cells are obtained directly from a donor, washed and implanted directly in combination with the polymeric material. The cells are cultured using techniques known to those skilled in the art of tissue culture.

Cell attachment and viability can be assessed using scanning electron microscopy, histology, and quantitative assessment with radioisotopes. The function of the implanted cells can be determined using a combination of the above-techniques and functional assays. For example, in the case of hepatocytes, *in vivo* liver function studies can be performed by placing a cannula into the recipient's common bile duct. Bile can then be collected in increments. Bile pigments can be analyzed by high pressure liquid chromatography looking for underivatized tetrapyrroles or by thin layer chromatography after being converted to azodipyrroles by reaction with diazotized azodipyrroles ethylanthranilate either with or without treatment with P-glucuronidase. Diconjugated and monoconjugated bilirubin can also be determined by thin layer chromatography after alkalinemethanolysis of conjugated bile pigments. In general, as the number of functioning transplanted hepatocytes increases, the levels of conjugated bilirubin will increase. Simple liver function tests can also be done on blood samples, such as albumin production. Analogous organ function studies can be conducted using techniques known to those skilled in the art, as required to determine the extent of cell function after implantation. For example, islet cells of the

pancreas may be delivered in a similar fashion to that specifically used to implant hepatocytes, to achieve glucose regulation by appropriate secretion of insulin to cure diabetes. Other endocrine tissues can also be implanted. Studies using labelled glucose as well as studies using protein assays can be performed to quantitate cell mass on the polymer scaffolds. These studies of cell mass can then be correlated with cell functional studies to determine what the appropriate cell mass is. In the case of chondrocytes, function is defined as providing appropriate structural support for the surrounding attached tissues.

This technique can be used to provide multiple cell types, including genetically altered cells, within a three-dimensional scaffolding for the efficient transfer of large number of cells and the promotion of transplant engraftment for the purpose of creating a new tissue or tissue equivalent. It can also be used for immunoprotection of cell transplants while a new tissue or tissue equivalent is growing by excluding the host immune system.

Examples of cells which can be implanted as described herein include chondrocytes and other cells that form cartilage, osteoblasts and other cells that form bone, muscle cells, fibroblasts, and organ cells. As used herein, "organ cells" includes hepatocytes, islet cells, cells of intestinal origin, cells derived from the kidney, and other cells acting primarily to synthesize and secret, or to metabolize materials.

Addition of Biologically Active Materials to the hydrogel.

The polymeric matrix can be combined with humoral factors to promote cell transplantation and engraftment. For example, the polymeric matrix can be combined with angiogenic factors, antibiotics, antiinflammatories, growth factors, compounds which

induce differentiation, and other factors which are known to those skilled in the art of cell culture.

For example, humoral factors could be mixed in a slow-release form with the cell-alginate suspension prior to formation of implant or transplantation. Alternatively, the hydrogel could be modified to bind humoral factors or signal recognition sequences prior to combination with isolated cell suspension.

Methods of Implantation.

The techniques described herein can be used for delivery of many different cell types to achieve different tissue structures. In the preferred embodiment, the cells are mixed with the hydrogel solution and injected directly into a site where it is desired to implant the cells, prior to hardening of the hydrogel. However, the matrix may also be molded and implanted in one or more different areas of the body to suit a particular application. This application is particlularly relevant where a specific structural design is desired or where the area into which the cells are to be implanted lacks specific structure or support to facilitate growth and proliferation of the cells.

The site, or sites, where cells are to be implanted is determined based on individual need, as is the requisite number of cells. For cells having organ function, for example, hepatocytes or islet cells, the mixture can be injected into the mesentery, subcutaneous tissue, retroperitoneum, properitoneal space, and intramuscular space. For formation of cartilage, the cells are injected into the site where cartilage formation is desired. One could also apply an external mold to shape the injected solution. Additionally, by controlling the rate of polymerization, it is possible to mold

the cell-hydrogel injected implant like one would mold clay.

Alternatively, the mixture can be injected into a mold, the hydrogel allowed to harden, then the material implanted.

Specific applications.

This technology can be used for a variety of purposes. For example, custom-molded cell implants can be used to reconstruct three dimensional tissue defects, e.g., molds of human ears could be created and a chondrocyte-hydrogel replica could be fashioned and implanted to reconstruct a missing ear. Cells can also be transplanted in the form of a thee-dimensional structure which could be delivered via injection.

The present invention will be further understood by reference to the following non-limiting examples.

Example 1: Preparation of a Calcium-Alginate-chondrocyte mixture and injection into mice to form cartilaginous structures.

A calcium alginate mixture was obtained by combining calcium sulfate, a poorly soluble calcium salt, with a 1% sodium alginate dissolved in a 0.1 M potassium phosphate buffer solution (pH 7.4). The mixture remained in a liquid state at 4°C for 30-45 min. Chondrocytes isolated from the articular surface of calf forelimbs were added to the mixture to generate a final cellular density of 1×10^7/ml (representing approximately 10% of the cellular density of human juvenile articular cartilage).

The calcium alginate-chondrocyte mixture was injected through a 22 gauge needle in 100 μl aliquots under the pannus cuniculus on the dorsum of nude mice.

The nude mice were examined 24 hours post-operatively, and all injection sites were firm to palpation without apparent diffusion of the mixture. Specimens were harvested after 12 weeks of *in vivo* incubation. On gross examination, the calcium alginate-chondrocyte specimens exhibited a pearly opalescence and were firm to palpation. The specimens weighed 0.11 ± 0.01 gms (initial weight 0.10 gms). The specimens were easily dissected free of surrounding tissue and exhibited minimal inflammatory reaction. Histologically, the specimens were stained with hematoxylin and eosin and demonstrated lacunae within a basophilic ground glass substance.

Control specimens of calcium alginate without chondrocytes had a doughy consistency 12 weeks after injection and had no histologic evidence of cartilage formation.

This study demonstrates that an injectable calcium alginate matrix can provide a three dimensional scaffold for the successful transplantation and engraftment of chondrocytes. Chondrocytes transplanted in this manner form a volume of cartilage after 12 weeks of *in vivo* incubation similar to that initially injected.

Example 2: Effect of cell density on cartilage formation.

Varying numbers of chondrocytes isolated from the articular surface of calf forelimbs were mixed with a 1.5% sodium alginate solution to generate final cell densities of 0.0, 0.5, 1.0, and 5.0 x 10^6 chondrocytes/ml (approximately 0.0, 0.5, 1.0, and 5.0% of the cellular density of human juvenile articular cartilage). An aliquot of the chondrocyte-alginate solution was transferred to a circular mold 9 mm in diameter and allowed to polymerize at room temperature by the diffusion of

a calcium chloride solution through a semi-permeable membrane at the base of the mold. The gels formed discs measuring 2 mm in height and 9 mm in diameter.

Discs of a fixed cellular density of 5 x 10^6 cells/ml were also formed in which the concentration of the sodium alginate and the molarity of the calcium chloride solutions were varied.

All discs were placed into dorsal subcutaneous pockets in nude mice. Samples were harvested at 8 and 12 weeks and examined for gross and histological evidence of cartilage formation.

Examinations of 8 and 12 week specimens revealed that a minimum cell density of 5 x 10^6 chondrocytes/ml was required for cartilage production which was observed only 12 weeks after implantation. On gross examination, the specimens were discoid in shape and weighed 0.13 ± 0.01 gms (initial weight 0.125 gms). The specimens were easily dissected free of surrounding tissue and exhibited minimal inflammatory reaction. Histologically, the specimens were stained with hematoxylin and eosin and demonstrated lacunae within a basophilic ground glass substance.

Cartilage formation was independent of calcium chloride concentration used in gel polymerization. Cartilage was observed in specimens with alginate concentrations varying from 0.5% to 4.0%; however, the lowest alginate concentration tested (0.5%) showed only microscopic evidence of cartilage.

Cartilage can be grown in a subcutaneous pocket to a pre-determined disc shape using calcium alginate gel as a support matrix in 12 weeks. Cartilage formation is not inhibited by either polymerization with high calcium concentrations or the presence of high alginate concentrations but

does require a minimum cellular density of 5×10^6 cells/ml.

The ability to create a calcium alginate-chondrocyte gel in a given shape demonstrates that it is possible to use this technique to custom design and grow cartilaginous scaffolds for craniofacial reconstruction. Such scaffolds have the potential to replace many of the prosthetic devices currently in use.

Example 3: Preparation of Implantable Premolded Cell-polymer mixtures.

250 µl aliquots of an isolated chondrocyte suspension was mixed with 750 µls of a 2% (w/v) sodium alginate solution (0.1 M K_2HPO_4, 0.135 M NaCl, pH 7.4). A 125 µl aliquot was placed into 9 mm diameter cell culture inserts with 0.45 µm pore size semipermeable membranes. The cell-alginate mixture was placed into contact with a 30 mM $CaCl_2$ bath and allowed to polymerize for 90 minutes at 37°C. After 90 minutes, the cell-alginate gel constructs were removed from the mold and had a diameter of 9 mm and a height of 2 mm. The discs were placed into the wells of 24-well tissue culture plates and incubated at 37°C in the presence of 5% CO_2 with 0.5 ml of a solution containing Hamm's F-12 culture media (Gibco, Grand Island, N.Y.) and 10% fetal calf serum (Gibco, Grand Island, N.Y.) with L-glutamine (292 µg/ml), penicillin (100 U/ml), streptomycin (100 µg/ml) and ascorbic acid (5 µg/ml) for 48 hrs.

Using this method, bovine chondrocyte-alginate discs were prepared, then implanted in dorsal subcutaneous pockets in athymic mice using standard sterile technique. After one, two, and three months, athymic mice were sacrificed, and the gel/chondrocyte constructs removed, weighed and

placed in appropriate fixative. The cell-polymer complexes were studied by histochemical analysis.

Cartilage formation was observed histologically after three months of *in vivo* incubation at an initial chondrocyte density of 5 x 10^6 cell/ml.

The above protocol was modified by using a range of $CaCl_2$ concentration and a range of sodium alginate concentrations. Cartilage formation was observed using 15, 20, 30, and 100 mM $CaCl_2$ baths and 0.5, 1.0, 1.5, 2.0, and 4.0% sodium alginate solutions.

By changing the mold within which the cell-alginate construct is created, the shape of the implant can be customized. Additionally, the mold need not be semipermeable as calcium ion can be directly mixed with the cell-alginate solution prior to being placed within a mold. The key feature is that the construct can be fashioned into a given shape prior to implantation.

Example 4: Preparation of injectable osteoblasts-hydrogel mixtures.

Using the methodology described above, bovine osteoblasts have been substituted for chondrocytes and injected into animals using a hydrogel matrix.

Histology after 12 weeks of *in vivo* incubation showed the presence of early bone formation.

Example 5: Use of the hydrogel matrix to form an immunoprotective matrix around the implanted cells.

By fashioning a cell-alginate construct as described above, one can use the hydrogel matrix to sterically isolate the encapsulated cells from the host immune system, and thereby allow allogenic cell transplants to form new tissues or organs without immunosuppression.

Bovine chondrocytes in an alginate suspension were transplanted into normal immune-competent

mice. Histology after six weeks of *in vivo* incubation shows the presence of cartilage formation. Gross examination of the specimens does not demonstrate features of cartilage. Literature states that similar chondrocyte xenografts without alginate do not form cartilage.

Modifications and variations of the compositions and methods of the present invention will be obvious to those skilled in the art from the foregoing detailed description. Such modifications and variations are intended to come within the scope of the following claims.

We claim:

1. A method for implanting tissue into an animal comprising

mixing a biodegradable, biocompatible hydrogel solution with dissociated cells and

implanting the mixture into the animal.

2. The method of claim 1 wherein the hydrogel solution is hardened prior to implantation in the animal.

3. The method of claim 1 wherein the hydrogel is injected into the animal as a cell suspension, which then hardens.

4. The method of claim 1 wherein the hydrogel is selected from the group consisting of alginate, polyphosphazines, polyethylene oxide-polypropylene glycol block copolymers, poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers.

5. The method of claim 4 wherein the hydrogel is hardened by exposure to an agent selected from the group consisting of ions, pH changes, and temperature changes.

6. The method of claim 5 wherein the hydrogel is hardened by interaction with ions selected from the group consisting of cations selected from the group consisting of copper, calcium, aluminum, magnesium, strontium, barium, tin, and di-, tri- or tetra-functional organic cations; anions selected from the group consisting of low molecular weight dicarboxylic acids, sulfate ions and carbonate ions.

7. The method of claim 4 wherein the hydrogel is further stabilized by cross-linking with a polyion.

8. The method of claim 1 wherein the cells are selected from the group consisting of chondrocytes and other cells that form cartilage, osteoblasts and other cells that form bone, muscle cells, fibroblasts, and organ cells.

9. The method of claim 1 wherein the hydrogel is molded to form a specific shape prior to implantation.

10. The method of claim 1 wherein the hydrogel is molded to form a specific shape after mixing with the cells and being implanted into the animal.

11. A composition for implanting tissue into an animal comprising

a hydrogel solution mixed with dissociated cells.

12. The composition of claim 11 wherein the hydrogel solution is hardened prior to implantation in the animal.

13. The composition of claim 11 wherein the hydrogel is injected into the animal as a cell suspension, which then hardens.

14. The composition of claim 11 wherein the hydrogel is selected from the group consisting of alginate, polyphosphazines, polyethylene oxide-polypropylene glycol block copolymers, poly(acrylic acids), poly(methacrylic acids), copolymers of acrylic acid and methacrylic acid, poly(vinyl acetate), and sulfonated polymers.

15. The composition of claim 14 wherein the hydrogel is hardened by exposure to an agent selected from the group consisting of ions, pH changes, and temperature changes.

16. The composition of claim 15 wherein the hydrogel is hardened by interaction with ions selected from the group consisting of cations selected from the group consisting of copper, calcium, aluminum, magnesium, strontium, barium,

tin, and di-, tri- or tetra-functional organic cations; anions selected from the group consisting of low molecular weight dicarboxylic acids, sulfate ions and carbonate ions.

17. The composition of claim 14 wherein the hydrogel is further stabilized by cross-linking with a polyion.

18. The composition of claim 11 wherein the cells are selected from the group consisting of chondrocytes and other cells that form cartilage, osteoblasts and other cells that form bone, muscle cells, fibroblasts, and organ cells.

INJECTABLE POLYSACCHARIDE-CELL COMPOSITIONS

Abstract of the Invention

Slowly polymerizing polysaccharide hydrogels have been demonstrated to be useful as a means of delivering large numbers of isolated cells via injection. The gels promote engraftment and provide three dimensional templates for new cell growth. The resulting tissue is similar in composition and histology to naturally occurring tissue. This method can be used for a variety of reconstructive procedures, including custom molding of cell implants to reconstruct three dimensional tissue defects, as well as implantation of tissues generally.

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below), or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: INJECTABLE POLYSACCHARIDE-CELL COMPOSITIONS

the specification of which (check one)

____ is attached hereto

__X__ was filed on April 30, 1993
as application Serial No. _____

____ and was amended on: _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	
(Number)	(Country)	(Day/Month/Year Filed)	
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of the application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	Status (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	Status (patented, pending, abandoned)

"Injectable Polysaccharide-Cell Compositions"
By: Keith T. Paige, et
Filed: April 30, 1993
DECLARATION

As named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John S. Pratt	29,476
James L. Ewing, IV	30,630
Patrea L. Pabst	31,284
Jamie L. Greene	32,467
Cheryl K. Zalesky	33,052
Dean W. Russell	33,452
Claudia R. Adkison	P36,979
Charles T. Simmons	35,359

Send Correspondence to: Patrea L. Pabst, Esq.
Kilpatrick & Cody
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309-4530

Direct telephone calls to: Patrea L. Pabst (404)815-6508, or
John S. Pratt (404)815-6367

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Keith T. Paige

Inventor's signature _____ Date _____

Residence 37 Perry Street, No. 1, Brookline, Massachusetts 02146

Citizenship United States

Post Office Address Same as above

Full name of second joint inventor (if any) Joseph P. Vacanti

Inventor's signature _____ Date _____

Residence 14 Woodside Road, Winchester, Massachusetts 01890

Citizenship United States

Post Office Address Same as above

SUBSTITUTE DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below), or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"Injectible Polysaccharide-Cell Compositions"
the specification of which (check one)

____ **is attached hereto**

__X__ **was filed on** April 30, 1993
as application Serial No. 08/056,140

____ **and was amended on:** _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	
(Number)	(Country)	(Day/Month/Year Filed)	
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of the application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	Status (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	Status (patented, pending, abandoned)

(patented, pending, abandoned)

As named inventor, I hereby appoint the following attorney(s) and agent to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John S. Pratt	29,476
James L. Ewing, IV	30,630
Patrea L. Pabst	31,284
Jamie L. Greene	32,467
Cheryl K. Zalesky	33,052
Dean W. Russell	33,452
Claudia R. Adkison	36,979
Charles T. Simmons	35,359
David W. Bradin	P37,783

Send Correspondence to: Patrea L. Pabst, Esq.
Kilpatrick & Cody
1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309-4530

Direct telephone calls to: Patrea L. Pabst (404)815-6508, or
John S. Pratt (404)815-6367

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Linda Griffith-Cima

Inventor's signature Linda Griffith Cima Date 2/28/94

Residence 28 Burlington Street, Lexington, Massachusetts 02173

Citizenship United States

Post Office Address Same as above

Full name of second joint inventor (if any) Anthony Atala

Inventor's signature _____ Date _____

Residence 14 LaGrange Street, Newton, Massachusetts 02167

Citizenship United States

Post Office Address Same as above

Full name of sole or first inventor Charles A. Vacanti

Inventor's signature _____ Date _____

Residence 5 Bushnell Drive, Lexington, Massachusetts 02173

Citizenship United States

Post Office Address Same as above

Full name of second joint inventor (if any) Keith T. Paige

Inventor's signature _____ Date 2/24/94

Residence 37 Perry Street, No. 1, Brookline, Massachusetts 02146

Citizenship United States

Post Office Address Same as above

Full name of sole or first inventor Charles A. Vacanti

Inventor's signature Charles A. Vacanti Date 2/23/94

Residence 5 Bushnell Drive, Lexington, Massachusetts 02173

Citizenship United States

Post Office Address Same as above

Full name of second joint inventor (if any) Keith T. Paige

Inventor's signature _____ Date _____

Residence 37 Perry Street, No. 1, Brookline, Massachusetts 02146

Citizenship United States

Post Office Address Same as above

(patented, pending, abandoned)

As named inventor, I hereby appoint the following attorney(s) and agent to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

John S. Pratt	29,476
James L. Ewing, IV	30,630
Patrea L. Pabst	31,284
Jamie L. Greene	32,467
Cheryl K. Zalesky	33,052
Dean W. Russell	33,452
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Send Correspondence to: Patrea L. Pabst, Esq.
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1100 Peachtree Street, Suite 2800
Atlanta, Georgia 30309-4530

Direct telephone calls to: Patrea L. Pabst (404)815-6508, or
John S. Pratt (404)815-6367

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Linda Griffith-Cima

Inventor's signature _____ Date _____

Residence 28 Burlington Street, Lexington, Massachusetts 02173

Citizenship United States

Post Office Address Same as above

Full name of second joint inventor (if any) Anthony Atala

Inventor's signature Anthony Atala Date 2-18-94

Residence 68 Pine ST, WESTON, Massachusetts 02193

Citizenship United States

Post Office Address Same as above

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Keith T. Paige and Joseph P. Vacanti		
Serial No:	08/056,140	Art Unit:	1806
Filed:	April 30, 1993	Examiner:	D. Adams
For:	INJECTIBLE POLYSACCHARIDE-CELL COMPOSITIONS		

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

CONSENT TO CORRECT NAMED INVENTORS

Sir:

Children's Medical Center Corporation represents that it is the owner of a joint interest in the above-identified application by virtue of an Assignment from Keith T. Paige and Joseph P. Vacanti executed on June 17, 1993 and recorded in the Patent and Trademark Office on July 6, 1993 at reel 6626, frame 0675, in U.S.S.N. 08/056,140, filed April 30, 1993 by Keith T. Paige and Joseph P. Vacanti; and by virtue of Assignments by Anthony Atala and Charles A. Vacanti executed on February 23, 1994 and included herewith.

The undersigned signatory, William New, states that he/she is empowered to act on behalf of Children's Medical Center Corporation, that he/she has reviewed the evidentiary documents establishing ownership of the above-identified application by Children's Medical Center Corporation,

and certifies that, to the best of his/her knowledge and belief, title is in Children's Medical Center Corporation.

As assignee of record of a joint interest in the above-identified application, consent is hereby granted to correct the named inventors to properly delete Joseph P. Vacanti and add Linda G. Cima, Anthony Atala, and Charles A. Vacanti, leaving Linda G. Cima, Anthony Atala, Charles A. Vacanti, and Keith T. Paige as the correctly named actual joint inventors.

The incorrect naming of Joseph P. Vacanti and failure to name Linda G. Cima, Anthony Atala, and Charles A. Vacanti in the above-identified application U.S.S.N. 08/056,140 filed April 30, 1993 was an inadvertent error made without deceptive intention on the part of Children's Medical Center Corporation.

CHILDREN'S MEDICAL CENTER CORPORATION

By: _____
Name: WILLIAM NEW
Title: DIRECTOR, RESEARCH ADMINISTRATION

Date: 3/15/94

CERTIFICATE UNDER 37 CFR 3.73(b)

Applicant: Keith T. Paige and Joseph P. Vacanti

Application No.: 08/056,140

Filed: April 30, 1993

For: INJECTIBLE POLYSACCHARIDE-CELL COMPOSITIONS

Children's Medical Center Corporation, a corporation of Massachusetts
(Name of Assignee) Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

certifies that it is the assignee of the entire right, title and interest in the patent application identified above by virtue of:

1. An assignment from Keith T. Paige and Joseph P. Vacanti executed on June 17, 1993, and recorded in the Patent and Trademark Office on July 6, 1993, at reel 6626, frame 0675, in U.S.S.N. 08/056,140, filed April 30, 1993; and by virtue of Assignments by Anthony Atala and Charles A. Vacanti executed on February 23, 1994, and included herewith.

Copies of the assignments are attached.

The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and believe are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : 3/14/94

Name : WILLIAM NEW

Title : DIRECTOR, RESEARCH ADMINISTRATION

Signature : [signature]

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Keith T. Paige and Joseph P. Vacanti		
Serial No:	08/056,140	Art Unit:	1806
Filed:	April 30, 1993	Examiner:	D. Adams
For:	INJECTIBLE POLYSACCHARIDE-CELL COMPOSITIONS		

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

PETITION UNDER 37 C.F.R. § 1.48

Sir:

As authorized under 37 C.F.R. § 1.48, the Applicants and the undersigned attorney respectfully request amendment of the above-identified application to delete Joseph P. Vacanti as a joint inventor and to add Linda G. Cima, Anthony Atala, and Charles A. Vacanti as joint inventors in the above identified application. It is further requested that the order of inventorship be as follows:

1. Linda G. Cima
2. Anthony Atala
3. Charles A. Vacanti
4. Keith T. Paige.

Appended hereto is a Statement of Facts verified by all the originally named inventors establishing that on April 30, 1993 an inadvertent error was made without deceptive intention by

incorrectly naming Joseph P. Vacanti as a joint inventor and by failing to name Linda G. Cima, Anthony Atala, and Charles A. Vacanti as joint inventors in the present application, U.S.S.N. 08/056,140. This error was first discovered in June 1993.

Since the discovery of the error, Applicants and the undersigned attorney have diligently proceeded to correct the named inventors to delete Joseph P. Vacanti and add Linda G. Cima, Anthony Atala, and Charles A. Vacanti.

The assignees, Massachusetts Institute of Technology and Children's Medical Center Corporation, have consented to the correction and order of the named inventors to delete Joseph P. Vacanti and add Linda G. Cima, Anthony Atala, and Charles A. Vacanti. Consents to Correct Named Inventors and Certificates Under 37 C.F.R. § 3.73(b) executed by the assignees are also appended hereto.

Also appended hereto is a Substitute Declaration and Power of Attorney executed by all the actual inventors and a check in the amount of $130.00 for the requisite fee under 37 C.F.R. § 1.17(h).

The appended (1) Statement of Facts; (2) Declaration executed by all actual inventors; (3) written consents of the assignees and Certificates Under 37 C.F.R. § 3.73(b); and (4) requisite fee are believed to satisfy the requirements to amend

the above-identified application to name the correct inventors as required pursuant to 37 C.F.R. § 1.48.

Accordingly, the Examiner is respectfully requested to grant this Petition to amend the above application to correctly name the correct inventors.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: March 16, 1994

KILPATRICK & CODY
1100 Peachtree Street, Suite 2800
Atlanta, GA 30309-4530
404/815-6508

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8a

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Patricia Holzer

Date: March 16, 1994

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Keith T. Paige and Joseph P. Vacanti		
Serial No:	08/056,140	Art Unit:	1806
Filed:	April 30, 1993	Examiner:	D. Adams
For:	INJECTIBLE POLYSACCHARIDE-CELL COMPOSITIONS		

Commissioner of Patents
and Trademarks
Washington, D.C. 20231

CONSENT TO CORRECT NAMED INVENTORS

Sir:

Massachusetts Institute of Technology represents that it is the owner of a joint interest in the above-identified application by virtue of an Assignment from Linda G. Cima executed on February 28, 1994 and included herewith in U.S.S.N. 08/056,140, filed April 30, 1993 by Keith T. Paige and Joseph P. Vacanti.

The undersigned signatory, Lita L. Nelsen, states that he/she is empowered to act on behalf of Massachusetts Institute of Technology, that he/she has reviewed the evidentiary documents establishing ownership of the above-identified application by Massachusetts Institute of Technology, and certifies that, to the best of his/her knowledge and belief, title is in Massachusetts Institute of Technology.

As assignee of record of a joint interest in the above-identified application, consent is hereby granted to correct the named inventors to properly delete Joseph P. Vacanti and add

Linda G. Cima, Anthony Atala, and Charles A. Vacanti, leaving Linda G. Cima, Anthony Atala, Charles A. Vacanti, and Keith T. Paige as the correctly named actual joint inventors.

The incorrect naming of Joseph P. Vacanti and failure to name Linda G. Cima, Anthony Atala, and Charles A. Vacanti in the above-identified application U.S.S.N. 08/056,140 filed April 30, 1993 was an inadvertent error made without deceptive intention on the part of Massachusetts Institute of Technology.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

By: Lita Nelsen
Name: LITA L. NELSEN, DIRECTOR
Title: TECHNOLOGY LICENSING OFFICE

Date: Mar 9, 1994

CI IFICATE UNDER 37 CFR 3.73,)

Applicant: Keith T. Paige and Joseph P. Vacanti

Application No.: 08/056,140

Filed: April 30, 1993

For: INJECTIBLE POLYSACCHARIDE-CELL COMPOSITIONS

Massachusetts Institute of Technology, a corporation of Massachusetts
(Name of Assignee) Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

certifies that it is the assignee of the entire right, title and interest in the patent application identified above by virtue of:

1. An assignment from Linda G. Cima executed on February 28, 1994 and included herewith in U.S.S.N. 08/056,140, filed April 30, 1993.

A copy of the assignment is attached.

The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and believe are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date : Mar 9, 1994

Name : LITA L. NELSEN, DIRECTOR
TECHNOLOGY LICENSING OFFICE

Title :

Signature : Lita Nelsen

The "Received" stamp of the Patent Office imprinted hereon acknowledges the filing of: 08/056140

Applicant: Keith T. Paige, et al.

Serial & Docket No. MIT6196

Filed: April 30, 1993 EXPMAIL NO. TB258352501US

Papers Submitted:

New patent application "Injectable Polysaccharide-Cell Compositions" (1 pg Abstract, 17 pgs. Spec., 3 pgs claims, unexecuted Declaration, check in the amount of $355.00, Authorization to charge Deposit OrderAccount, Express Mail Transmittal letter, fee sheet(in duplicate)

Date: April 30, 1993

By: Patrea L. Pabst, Reg. No. 31,284